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Setting up of beef aging processes and comparison of two methods: Traditional Dry Aging and a novel method with High Permeable to Water Vapour Bag

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Abstract

The objective of this experiment was to compare traditional dry ageing of beef with a novel procedure with a highly permeable to moisture. Six similar pieces of whole short sirloin from the same anatomic side of crossbreed Holstein x Angus were selected to conduct this experiment for 28 or 45 days at a Temperature of 1.0 ± 2.0 ºC and Relative Humidity of 78 ± 20% as ageing conditions. There were found differences ($P < 0.0001$) between ageing methods on yield parameters as weight losses from 0 and 28th day and on the estimated total ageing time (0 – 45 d). Defrost losses showed to be influenced by ageing method at 28 days ($P < 0.0002$), as well as cooking losses were affected by the interaction of time and ageing method ($P < 0.0001$), these ones related to Water Holding Capacity. Ageing time also affected CIE-Lab scale values that fluctuated among ageing period ($P < 0.0001$). Texture parameters, measured by Shear Force test, were influenced ($P < 0.0001$) by ageing period. Some sensory traits are slightly but statistically ($P < 0.05$) influenced by the interaction of time and ageing method. Dry ageing in a highly permeable to water vapour bag showed differences with traditional dry ageing, affecting production and saleable yields, as well as being an influencing factor on quality traits that may affect consumer perception in election of such a premium product as aged beef.

Resumen

El objetivo de este experimento ha sido el de comparar la maduración tradicional de carne con un novedoso procedimiento con una bolsa altamente permeable a la humedad. Seis piezas de similares características de un lomo bajo del mismo costado anatómico de novillas cruza Holstein x Angus fueron seleccionados para llevar a cabo este experimento por 28 o 45 días a una temperatura de 1.0 ± 2.0 ºC y humedad relativa de 78 ± 20% como condiciones de maduración establecidas. Han sido encontradas diferencias estadísticas ($P < 0.0001$) entre métodos de maduración en parámetros de rendimiento como pérdidas de peso de 0 a 28 días y en el periodo total estimado (0 – 45 días). Las perdidas por descongelación mostraron ser influenciadas por el método de maduración a los 28 días ($P < 0.0002$), así como las perdidas por cocción fueron afectadas por la interacción del tiempo y tipo de maduración ($P < 0.0001$), éstas relacionadas a la capacidad de retención de agua. El periodo de maduración también ha afectado valores de la escala CIE-Lab ($P < 0.0001$) que fluctuaron entre tiempos de maduración. Los parámetros de textura, medidos por Shear Force Test, fueron influenciados por los periodos de maduración ($P < 0.0001$). Algunas características sensoriales han sido estadísticamente influenciadas ($P < 0.05$) por la interacción entre el periodo y el método de maduración. La maduración en seco en una bolsa altamente permeable a la humedad ha mostrado diferencias con la maduración tradicional en seco, afectando rendimientos productivos y de venta, así como siendo un factor de influencia en parámetros de calidad que podrían afectar la percepción del consumidor en la elección de un producto selecto como la carne madurada.

CONTENT

1 INTRODUCTION

1.1 Overview

Ageing processes in beef are associated with the development of enhanced characteristics, as increased tenderness, flavour and overall palatability (Campbell et al., 2001). By its nature, beef ageing can be easily described as procedures that allows the natural enzymatic and biochemical processes that results on the enhancement of various parameters as the tenderness, the development of desirable flavours and many others factor that increase the willingness to buy these products.

1.2 Beef ageing methods

1.2.1 Traditional ageing. Dry aged beef

These procedures can be done on beef carcasses, primals and subprimals, whereby are stored with or without packaging. This is in fact what defines the type of drying process. As shown in Figure 1, traditional dry ageing, consists of putting a piece of beef under refrigeration temperatures and relative humidity for a period of time to allow the biochemical and physicochemical changes that results in the "unique flavour that can´t be reached by the others beef ageing procedures" (Campbell et al., 2001).

Wet ageing, which consist of ageing meat in a sealed barrier package at refrigerate temperature, and the most recently method described as ageing in a highly moisture-permeable bag, a novel kind of bag technology that has a highly water vapor transmission rate that have been introduced to the market. "Dry ageing" in this bag would produce dry-aged-flavour like achieved with traditional dry ageing conditions. Ahnström et al.(2006) noted that the material in the bag works as a permeable plastic and designed to decrease weight, trim loss and/or material contamination, and increasing yield and leading to similar tenderness and other sensory attributes.

a.

b.

c.

Figure 1. a. Dry ageing b. Wet ageing c. Ageing in a high moisture permeable bag. Source: a. IRTA; b. http://forum.bradleysmoker.com/index.php?topic=24543.0; c. IRTA

1.2.2 Wet ageing

In the 1960s, when the vacuum packaging became an alternative on the managing and transport of beef by the first time in the United States, and later internationally, it became the leading ageing method. As the usage of vacuum packaging was accepted and lead to development of new techniques, by the 1980s it became the main way used to handle and trade beef up to 90% of all the marketed beef (Savell, 2008). Clearly, due to the advantages offered by this new way of storing and ageing beef (as the reduction of shrinkage, less trimming losses) vacuum packaging became more popular for both parts of the supplying chain, the processor and the retailing services, and the foodservice sectors and markets (Savell, 2008).

Minks and Stringer (1972) proved the advantages of vacuum packaging compared to dry ageing when considering shrinkage and shell-life yields, without losing desirable palatability characteristics. Therefore, the growth of the vacuum bag ageing, transformed into subprimals ready to retail, made the dry ageing process a minor contributor in this growing market.

1.2.3 Novel procedures. Dry ageing in a highly permeable to water vapour bag

In more recently studies, a completely new concept regarding beef ageing has been described (Ahnström et al., 2006). Such method allows the combination of the benefits of dry ageing and wet ageing processes. These benefits are less trimming loses, a high diminution of the shrinkage rates, as the own development of flavours and organoleptic characteristics. The highly permeable to water vapour bag offers the possibility of obtaining significant yield differences compared to the traditional dry ageing. In fact, researchers such as Smith et al., (2014), showed that retail cutting tests in dry-aged subprimals experienced lower total saleable yield than wet-aged subprimals. These parameters are not only affected by ageing, thus to the cutting and preparing of saleable products (trimming losses and manufacturing processes are involved). Above all, Table 1 presents a comparison between ageing methods from the standpoint of the novel method that has been described.

Table 1. Advantages comparison between ageing methods

	Dry ageing	Wet ageing	Permeable water vapour bag	Reference
Physical properties	High trimming loss	Less/non-humidity losses (minimum weight losses)	Less humidity losses compared to dry ageing	(Ahnström, Seyfert, Hunt, & Johnson, 2006b; Berger et al., 2018; Campbell et al., 2001)
	High ratio of weight loss due to humidity losses		Enhanced enzymatic tenderization	
Sensory parameters	Development of characteristic flavours	Non-significant differences in flavour between methods	Reduced microbial spoilage (bacteria and yeast)	(Ahnström et al., 2006b; Dashdorj, Tripathi, Cho, Kim, & Hwang, 2016
	High overall liking	Higher juiciness compared to dry ageing	Development of dry-aged-like flavours	

1.3 Quality of meat. Meat quality traits that affect ageing processes

There are studies that reflect the importance of certain factors that can affect the quality of the meat to be aged (Brooks et al., 2000; Warren & Kastner, 1992) such as post-mortem proteolysis, intramuscular fat (marbling), connective tissue (Brooks et al., 2000). Proteolysis is the predominant influencing factor that influences in the development of meat tenderization. The range and extent of the response to ageing and following tenderization are dependent of factor such as species, animal age, diet, breed, type of muscle, marbling characteristic and ageing conditions (Kim et al., 2018).

Another important factor related to beef tenderization is the ionic strength that is responsible of protein solubilisation that affects consistently on thickness of myofilaments that changes the nature of actin/myosin bond and the subsequent weakening of this interaction. The myofibrillar structure (Figure 2) is clearly altered in this tenderization progress by proteases in post-mortem beef ageing. These structures are not affected by proteolysis until 3-4 days post-mortem. Proteases (calcium dependent proteases or calpain), are activated by a Ca^{2+} and reproduces post-mortem changes in myofibrils and are associated to meat tenderization (Wu & Smith, 1987). Therefore, the tenderness of meat is dependent of weakening and alteration of myofibrillar structures and has been largely attribute to the proteolytic enzymes. Thus, the calpain system influences post-mortem proteolysis. This system has an important role in influencing tenderness of meat and represents a meat quality marker (Kemp et al., 2010)

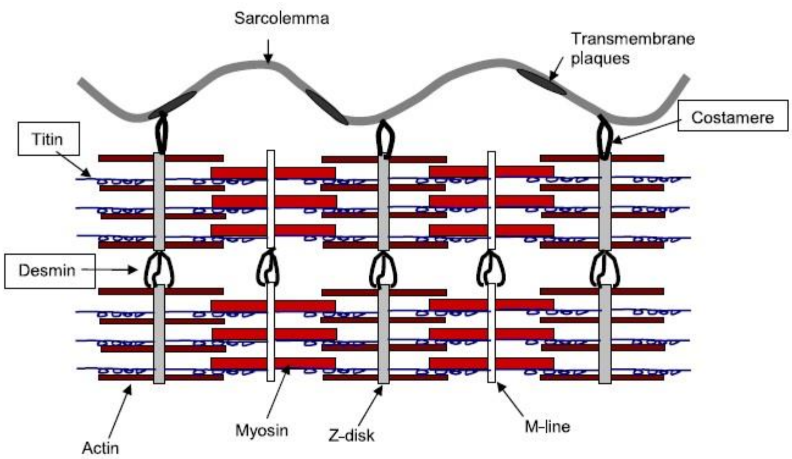


Figure 2. Myofibrillar structure of meat. Source: adapted by Kemp et al. (2010)

The thickness of subcutaneous fat plays an important role in reducing the shortening damage caused during the cooling process and dry ageing. Between the processes of slaughtering and rigor mortis, series of biochemical changes involve sarcoplasmic reticulum and the binding of calcium, as well as the presence of ATP that contracts the muscle at a maximum level, which may reduce the meat tenderness. Thus, the increase in the thickness of subcutaneous fat improves meat tenderness, allowing the carcass to chill slowly and preventing the quoted damage (Smith et al., 2014).

1.4 Microbiology

Concerning about microbiology, dry ageing involves restricting bacterial growth and encourages beneficial mould growing. During the process of beef dry ageing, there could be moulds occurring. *Thamnidum* genre (Figure 3) that could even be desirable because their enzymes are able to penetrate the meat and create collagenolytic enzyme that breaks down the muscle and connective tissues, leading to the improvement of tenderness and taste ("PrimeSafe - Ageing of Beef," n.d.). In addition, concerning about food safety, *Rhizopus* and *Mucor* mould genera are other associated with dry ageing, however, they have been linked to human infections and could lead to undesirable meat characteristics ("PrimeSafe - Ageing of Beef," n.d.)

Figure 3. Dry aged beef with presence of mould, including *Thamnidium* genre. Source: http://cookingcoach.club/category6/Thamnidium-meatless-recipes.html

Dry aged steaks had a higher aerobic plate counts compared to the control of a study conducted by Campbell et al. (2001). The duration of dry ageing did not affect aerobics count, maybe due to the growth inhibition caused by surface drying and storage temperatures low enough to delay growth. However, dry ageing relies on the reduction of the amount of water activity on the surface and minimizes the bacterial growth (DeGeer et al., 2009) revealed that novel dry ageing bags would not have significant differences in *E. coli*/coliforms and lactic acid bacteria microbial growth than that of traditional dry ageing.

1.5 Normative frame

According to the European Union (UE) laws, there is a big variation according to food safety requirements between countries and also between the regions of countries. Therefore, the maximum authority of the UE with respect to Food Safety, establishes the requirements that must comply in order to ensure the quality and safety of these products (Commission regulation (EC) No 2073/2005 of 15 November 2005 on Microbiological Criteria for Foodstuffs, 2007). In Spain, the BOE Real Decreto 474/2014 defines the aged beef (regardless of the ageing method) as subproduct and the main concerning topics about the subject as retailing, transporting and handling conditions.

1.6 International guidelines

Different guidelines for beef ageing have been published in different countries. In 2013, U.S. Meat Export Federation published the Guidelines for U.S. Dry-Aged Beef for International Markets. In Belgium, the supplementary chapter entitled *Maturation longue à sec ou «dry-ageing »* was first published in 2014 and modified in 2017; and most recently, in 2017 Australia published PrimeSafe - Ageing of Beef.According to "PrimeSafe - Ageing of Beef," n.d., the requirements to ensure the production and saleable of dry aged beef are enclosed to a range of temperature between -0.5º C to 1º C, the Relative Humidity (RH) must be between 75 and 85% and the air flow velocity between 0.2 to 0.5 m/s. In case of wet ageing the temperature must be below 5º C and the product must be tested for shelf life including *Enterobacteriaceae* and *E. coli* at the end of the ageing period (Table 2).

Table 2. Comparison of suggested range between international systems and MatureMeat® recommendation.

Source	Suggested Range for dry ageing Storage temperature (ºC)	Relative Humidity (%)	Air flow (m/s)	Suggested range for wet ageing Storage temperature (ºC)
PrimeSafe - Ageing of Beef – Australia	0.5 -1	75 - 85	0,2 - 0,5	< 5º
Guidelines for U.S. Dry-Aged Beef for International Markets: U.S. Meat Export Federation	0 - 4	80 - 85	0,5 - 2	-
MaturMeat ® recommendations – Italy	1	78	n.d.	-

On the other hand, in the document "Guidelines for U.S. Dry-Aged Beef for International Markets : U.S. Meat Export Federation," n.d., describes that the range of temperature for ageing beef must remain between 0º C to 4º C and the RH 80 to 85%, and values between 0,5 to 2 m/s of air flow velocity. These factors can affect in both ways, when the values are too low or excessive. This could be seen in excessive microbial growth (resulting in product spoilage) when temperature and RH are too high, or even the ageing ceases when the meat is frozen; and there could be excessive weight and trim losses when temperature is too low and air flow values too high. In addition, possible spoilage of the product could be reached by the excessive growing of bacteria when airflow velocity is too low.

1.7 Aged beef retailing

Dry ageing of subprimals at the retail level can enhance overall palatability while creating a premium price for these products and decreasing any variation of sensory characteristics due to slaughter plant location and many other intrinsic factors (Miller et al., 1997). Due to the high costs linked to storage, shrinkage and trimming loses, not so many restaurants or specialized butcher stores offer dry aged beef, leading to identify an important market niche. Furthermore, market's needs and the consumer expectation are needed to identify ways to improve meat organoleptic characteristics and reach a suitable product. (Smith et al., 2014).

1.8 Objectives

We hypothesised that meat ageing in a high permeable bag could have advantages compared to the dry traditional ageing method. The present work aims to study a comparison of the dry ageing (DA) and novel ageing in highly permeable to water vapour bag (TB), as well the assessment of temperature and relative humidity in a commercial ageing chamber. This objective will be reached by the comparison of the two methods, studying their effects on yield, instrumental measurements and organoleptic characteristics of subprimals submitted to this process.

2 METHODOLOGY

2.1 Experimental design

Two type of ageing methods were studied during two ageing methods in two periods: 28 and 45 days, in a 2 x 2 factorial design (Table 3). Treatments consisted of traditional dry ageing (hereafter, DA) and dry ageing in the novel bags (named Tublin bag, hereafter, TB named Tublin bag) for 28 and 45 days as well.

Table 3. Treatments distribution and number of samples

	Methods	
Ageing period	**DA**	**TB**
28 days	6	6
45 days	6	6

Six units of short sirloin of the same anatomic side of Holstein breed crossed with Angus breed animals were purchased from a commercial retailer and delivered at IRTA Monells (Monells, Girona). Each short sirloin was cut in two parts according to Figure 4.

Figure 4. Sample manufacturing; short sirloin cutting.

The short sirloins were divided laterally in two, so each part provided two 24 cm length samples approximately that were weighed to prepare the main primals to be transformed in the main experimental units. Half of the total of samples were assigned to TB treatment and prepared on a way to ensure the right performance of it. Samples were deboned and cut into subprimals by an experimented butcher. Treatment combination were assigned randomly to each loin section and managed by the following scheme (Figure 5).

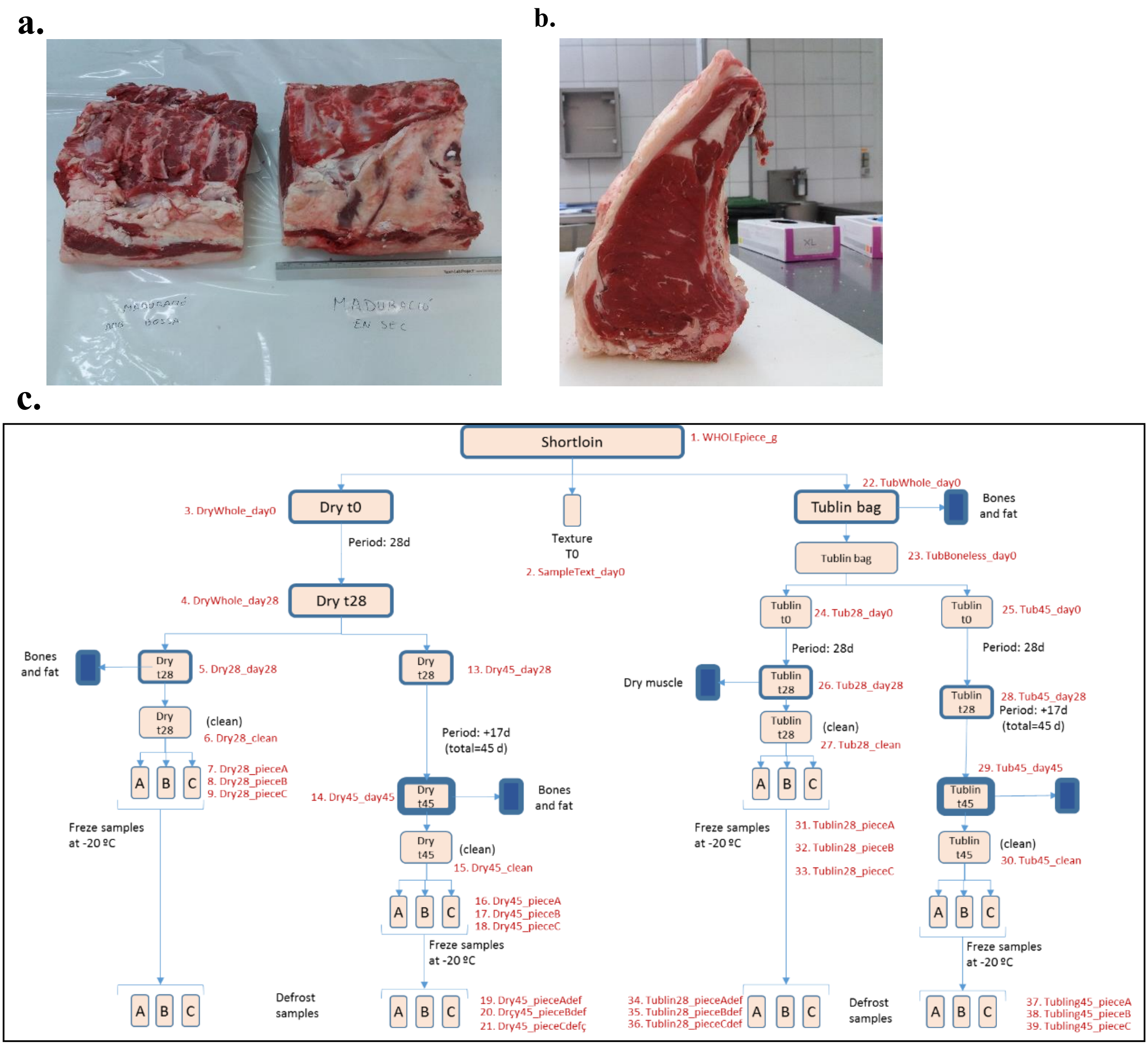


Figure 5. Sample preparation. a. TB and DA samples; b. DA sample; c. Cutting and weighing scheme

2.1.1 Dry ageing process

The samples corresponding to Dry Ageing treatments were placed into the MatureMeat ® chamber under 1 ºC and 78% of temperature and relative humidity respectively. Samples were monitored constantly to make sure there was not mould growing or any external font of variability (Figure 6).

Figure 6. Dry ageing (DA) sample

2.1.2 Ageing with a highly permeable to water vapour bag (Tublin ® Bag)

The samples in novel dry ageing bags (Figure 7) were deboned and vacuum packaged, into bags with the following characteristics (TUBLIN 10, Denmark):

- 2.0 mil thermoplastic elastomer made of flexible polymer and rigid polyamide
- water vapour transmission rate 8000 g/15l /m2/24 h at 38 C and 50% relative humidity;
- oxygen trans-mission of 2.3 mL/m2/d at 38 C and 50% relative humidity;

The main characteristic of these novel bags is that they have a greater rate of water vapour exchange from the surface of the meat to the atmosphere, thus simulating conditions of dry ageing.

Figure 7. Samples packaged into highly moisture permeable bags (TB treatments)

2.2 Ageing chamber (MaturMeat®)

A new ageing chamber (MATURMEAT ®, Arredoinox, Milano, Italy) has been used for this study (Figure 8). Maturmeat® is an ageing chamber that consists on a refrigeration device that allows to program various types of processes, including dry ageing beef. There are many pre-set programs with specific conditions that let running the equipment, and in the same way, the operator can customize the conditions of ageing, changing configuration for temperature and relative humidity.

The range of working conditions of the chamber are: temperatures, between -3 ºC to 35 ºC, and relative humidity rates from 30% to 99%. An inner control system sets the relative humidity by the injection of water vapour until a pre-set percentage is reached, and the control system records these variables in order to have a control of the parameters and access to information for the decision take and consider actions to be taken. The following parameters were set for this experiment (Table 4).

Table 4. Set conditions in ageing chamber

Nominal temperature (ºC)	Relative Humidity (%)	Air flow (m/s)
1.0 ºC ± 2.0 ºC	78	1 – 1.5 m/s

a.

b.

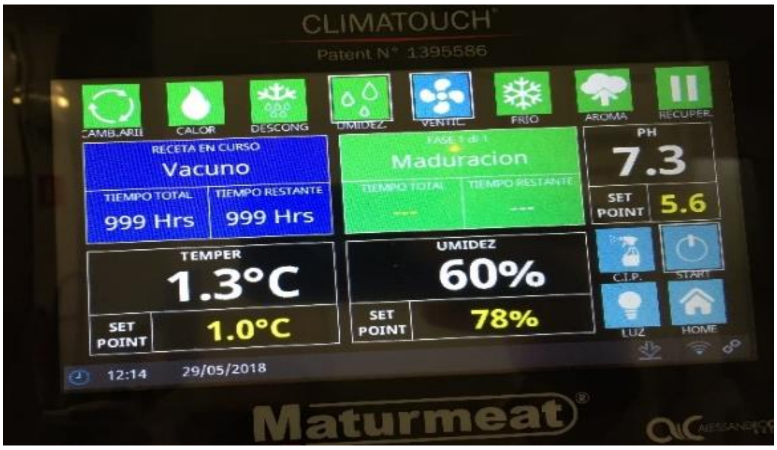


Figure 8. a. MatureMeat® ageing chamber with samples b. Established conditions on MatureMeat® display panel

2.2.1 Thermic profile of ageing chamber

As part of the description of the ageing process, the thermic profile of the meat chamber was characterised with the objective to identify any difference between the pre-set conditions of the MatureMeat (R) chamber and the real conditions, in terms of temperature (ºC) and Humidity (%).

For this study 6 data loggers were used. These data loggers were set up to record atmosphere temperature (ºC) and relative humidity (%) every minute during a period of 24h. After each cycle of 24h, data loggers were reallocated to a new position according to the scheme showed in Figure 9. After 9 days period, the temperature and relative humidity was recorded in 45 different points ensuring the maximum outreach. One data logger was used as reference and therefore, was always placed in the same position (position 14) which was a central point of the machine to have a unique reference (Figure 9).

Table 5. Main traits of the data loggers used for this study

Data logger	Measuring range	
	T °C	% RH
Rotronic hygrolog HL NT	/ 0_+50	0_100
Rotronic hygrolog HL 20	/ -10_60	0_100
Testo 177 H1	/-20_+70	0_100
Testo 177 H1	/-20_+70	0_100
Testo 177 H1	/-20_+70	0_100
Testo 177 H1	/-20_+70	0_100

The data obtained with the different temperature sensors at each measured coordinate were processed using an application developed with the computing MATLAB® computer package, based on the Delaunay Triangulation as a method of interpolation of values in 3D.

The registers in the reference points (coordinate number 14) and the experimental registers of the different coordinates on a determined cycle allowed the application to estimate the temperatures in the same coordinates in cycles where there was no experimental record.

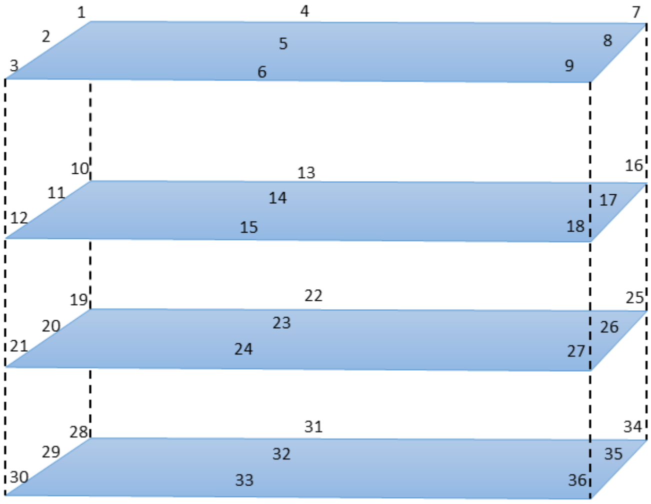


Figure 9. Data loggers position on ageing chamber

2.3 Physical measurements on the meat

2.3.1 Samples preparation

After each ageing period (28 or 45d), meat was taken out of the ageing chamber for the sample preparation. After 28 day of ageing, samples of two treatments where weighed and registered each length dimensions to compare with the initial data to pursue a weight loss and shrinkage rate respectively.

In the case of traditional dry ageing samples, 10 cm of the short sirloins primals were cut and the rest of the short sirloin was introduced again in the ageing chamber until the end the second ageing period (45d).

The 10cm-piece (aged only 28d) was deboned and trimmed to remove dry and discoloured portions. For the TB method, samples were also trimmed to remove dry and discoloured portions.

From the remaining meat, the following samples were taken:

- 2.5 cm width sample for the shear force test. On the surface, instrumental colour analyses was performed after 30 minutes blooming.
- 2.5 cm width sample for the sensory assessment
- 2.5 cm width sample for the training of the sensory panel

Same sampling protocol was followed for 45 days treatments (traditional and novel). Each weight of the samples was registered in every step, as well as the lefts from the trimming procedures.

Figure 10. Sample manufacturing. Trimmed sample. a. Texture analysis; b. Sensory analysis; c. Panel training

2.3.2 Weight measurements

Every whole short sirloin was weighed at the arrival to the centre and held in cold temperature (4º Celsius) before being manipulated and cut into subprimals to constitute the two main types of sub-primals for the methods of this study, Dry Ageing (DA) and Tublin bag Ageing (TB). The preparation of samples consisted of preparing the sub-primals of similar conditions of weight (Figure 10).

Each cut was weighed (Figure 11) before and after each ageing process, before and after deboning and trimming, and after cutting each sample, as described in Table 6.

Table 6. List of weights that were recorded for each sample during the ageing process for 28 and 45 days.

DA samples	TB ageing samples
Whole piece at day 0	Whole piece at day 0
Whole piece at 28th day	Whole deboned piece
Subprimals at 28th d	Subprimals at day 0
45 d subprimals at 28th d	28 days aged subprimal
Subprimals at 45th day	45 days aged subprimal
Samples after trimming process	Samples after trimming process
After trimming lefts	After trimming lefts

Weight loss was measured by the difference between initial and final weight, in percentage points (x 100). In addition, trimmed tissue was weighted, and the trim losses are presented in percentage points.

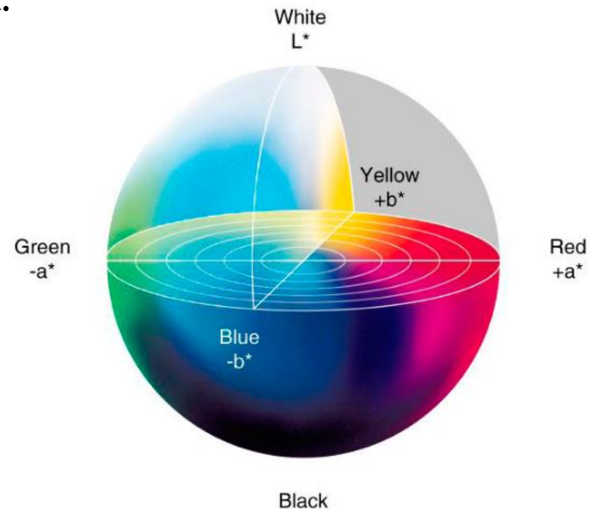
Figure 11. Samples weight measurements.

2.4 Meat quality measurements

2.4.1 Colour assessment

Instrumental colour was measured with a Spectrophotometer MINOLTA CR300 (Minolta Camera Corp., Meter Division, Ramsey, NJ) in the CIELAB SPACE (L*, a*, b*) with a closed cone diameter of 0.8 cm (Figure 12). The instrument was calibrated with a white tile using illuminant D65 with 10 degrees of standard observer. Each data point was the average of 3 measures carried out on different points of the surface of the meat sample. L* (degree of lightness), a* (degree of redness), b* degree of yellowness) were recorded.

a.



b.

Figure 12. a. CIELAB Space. b. Colour measurements with MINOLTA CR300 Spectrophotometer. Image source: a. https://www.grafigata.com/gestire-colouri-nella-stampa/ b. IRTA

For colour testing, there were used samples of each prepared for texture at day 0, and samples of each period.

2.4.2 Instrumental texture

For the shear force test and as is shown in Figure 13, after thawing for 36h at -18 to - 20 ºC, each 2.5 cm-thick steak was analysed with a Warner-Bratzler test. The analyses were performed with a Texture analyser Alliance RT/5 (MTS Systems Corp., Eden praire, MN, USA) equipped with a Warner-Bratzler blade with crosshead speed set at 2mm/s.

Each steak was cooked with a Rational SelfCooking Center oven until an internal temperature of 70 ºC was reached, then cut with an Ø12 mm cork borer, parallel to the muscle fibres, into six cross-sections of approximately one cm^2. Each cross-section was sheared using a Warner-Bratzler blade to calculate the shear force value and the toughness, in other words the amount of energy required to break the sample (Honikel, 1998).

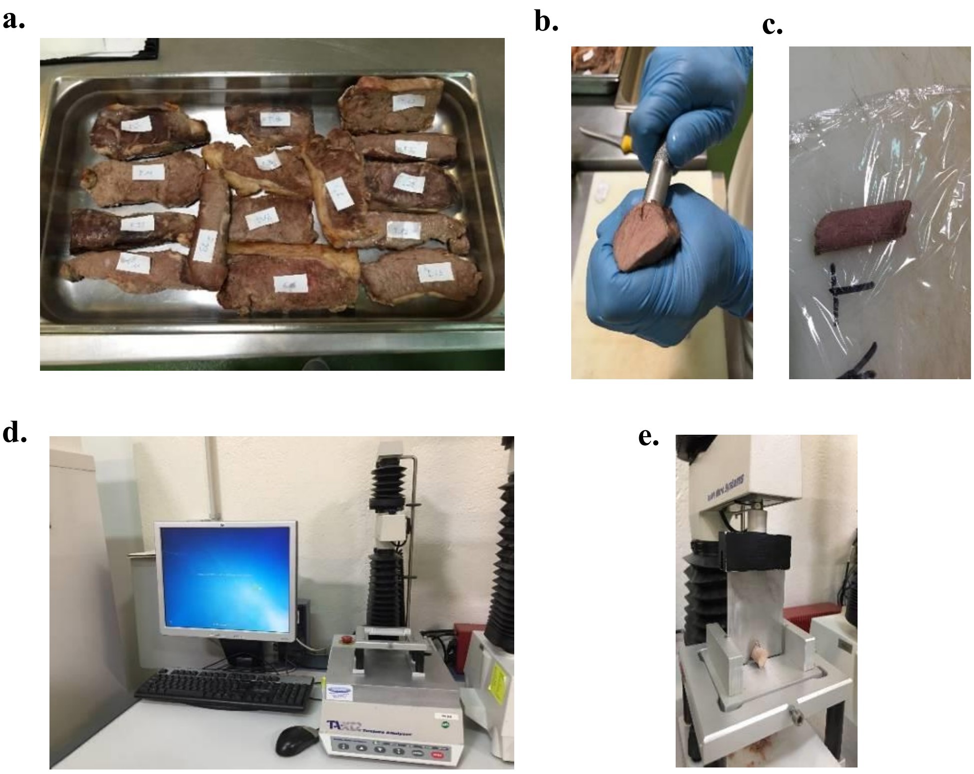


Figure 13. Shear force test flow. a. Samples (DA&TB) after cooking; b. Cork borer cutting parallel to muscle fibres; c. Subsample d. Texture analyser Alliance RT/5; e. Subsample submitted to Shear Force test.

2.4.3 Thawing loses

Subprimals were kept frozen at -20º Celsius in a fridge. Subprimals assigned for texture measurements were defrosted before being manipulated, in a 0 – 4 º Celsius (3ºC ± 1) during a period of 24hs. After defrosting samples were weighed and lead for next purposes. The percentage of thawing loses can be estimated by the following formula:

$$\textbf{Thawing loses: } \frac{(W2-W1)}{W1} x100$$

Where:

W_2: Weight of samples after defrosting

W_1: Weight of samples before freezing just after fabrication

2.4.4 Cooking losses

The samples that have been used for texture analysis were weighed before and after cooking to determine cooking loses. There were used a formula to determine the percentage of losing due to cooking (Figure 14).

$$\textbf{Cooking loses: } \frac{(Wc-W2)}{W2} x100$$

Where:

W_C: Weight of samples after cooking for 15 minutes at 70º Celsius

W_2: Weight of samples after defrosting

Figure 14. Weighing of samples after being cooked

2.4.5 Colour Preference and Purchase decision

Colour preference as purchase decision were measured by the trained panel by following the scale showed in Table 7 in samples at 28th day and 45th day of both ageing methods. The obtained punctuation was analysed looking for possible significant differences.

Table 7. Trained panel evaluation ratings for Dry Aged and Aged in Highly permeable water vapour bag samples.

Purchase decision	Colour preference	Punctuation
Definitively not buying	Very undesirable	1
Probably not buying	Moderately undesirable	2
Dubious buying	Slightly undesirable	3
Probably buying	Moderately desirable	4
Definitively buying	Very desirable	5

Trained panel scores (n=8) were based on a five-point hedonic scale where 1=worse and 5=best punctuation

2.4.6 Sensory analysis

2.4.6.1 Cooking of samples

Samples were kept in a refrigerated chamber at -18 to -20º C by 36 hours to defrost them. Right before cooking they were exposed at room temperature and proceeded with the cooking when arrived at a established temperature of 18º C to 20º C. A conventional both-sided grill was used until samples reached 40º C and blazed with a torch, to ensure the cooking of the edges in every side of the steaks. Once samples were completely sealed in every face of its surface, they were cooked to a final temperature of 63º C in a “ClassA” convection oven. While the whole process of cooking, samples were monitored using Testo 177 H1 fitted with a type-T thermocouple.

Samples where cut following the scheme represented at Figure 15 and placed in metallic containers covered with aluminium foil to keep all flavours inside, and were immediately served to the trained panellist in individual booths under red lights, following the distribution of the treatments showed in Table 8.

a.

b.

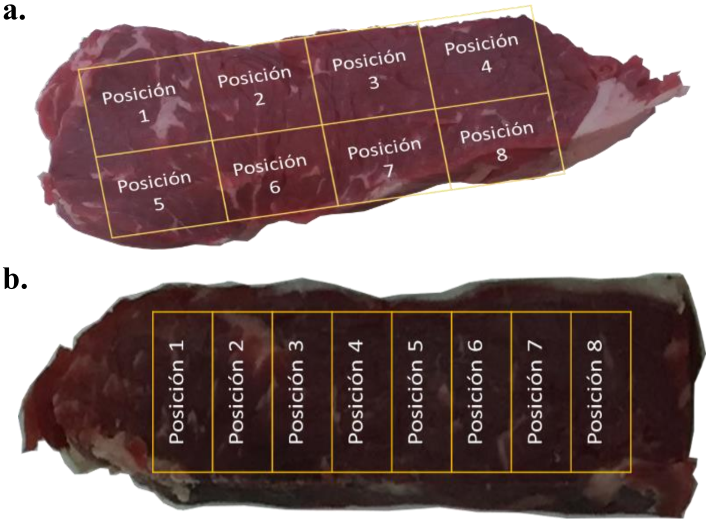


Figure 15. Positions of sensory subsamples for DA and TB samples

Table 8. Distribution of treatments and sensory sessions

		Cooking order			
Session	**N. ANIMAL**	**1st cooking**	**2nd cooking**	**3rd cooking**	**4th cooking**
1	**1**	Tublin 45d 131	Tublin 28d 274	Dry 45d 379	Dry 28d 815
2	**2**	Tublin 28d 140	Dry 28d 322	Tublin 45d 536	Dry 45d 831
3	**3**	Dry 45d 152	Tublin 45d 346	Dry 28d 554	Tublin 28d 875
4	**4**	Dry 28d 158	Dry 45d 352	Tublin 28d 639	Tublin 45d 947
5	**5**	Tublin 45d 186	Dry 28d 369	Tublin 28d 785	Dry 45d 982
6	**6**	Dry 28d 192	Dry 45d 376	Tublin 45d 787	Tublin 28d 986

The cooked subsamples were offered to the panellist following the distribution showed at Table 9 in order to ensure that each subsample from the same position of the different samples and avoid the effect of the position and direction of the muscle fibres. Subsamples were given to the trained panellists in a preheated pot to avoid temperature dissipation during handling.

Table 9. Distribution of subsamples offered to each panellist during sense analysis, based on McFie (1998) distribution.

	Panellist							
Session	**Panellist 1**	**Panellist 2**	**Panellist 3**	**Panellist 4**	**Panellist 5**	**Panellist 6**	**Panellist 7**	**Panellist 8**
1	Position 1	Position 2	Position 3	Position 4	Position 5	Position 6	Position 7	Position 8
2	Position 8	Position 1	Position 2	Position 3	Position 4	Position 5	Position 6	Position 7
3	Position 7	Position 8	Position 1	Position 2	Position 3	Position 4	Position 5	Position 6
4	Position 6	Position 7	Position 8	Position 1	Position 2	Position 3	Position 4	Position 5
5	Position 5	Position 6	Position 7	Position 8	Position 1	Position 2	Position 3	Position 4
6	Position 4	Position 5	Position 6	Position 7	Position 8	Position 1	Position 2	Position 3

Cooking and servicing of samples process is showed in Figure 16. Due to the timing of the trial, the sensory panelling was separated in two main days (consecutive days). This meant that the panel composition varied because the absent of one trained panellist and the data analysis was undertook only under common panellist.

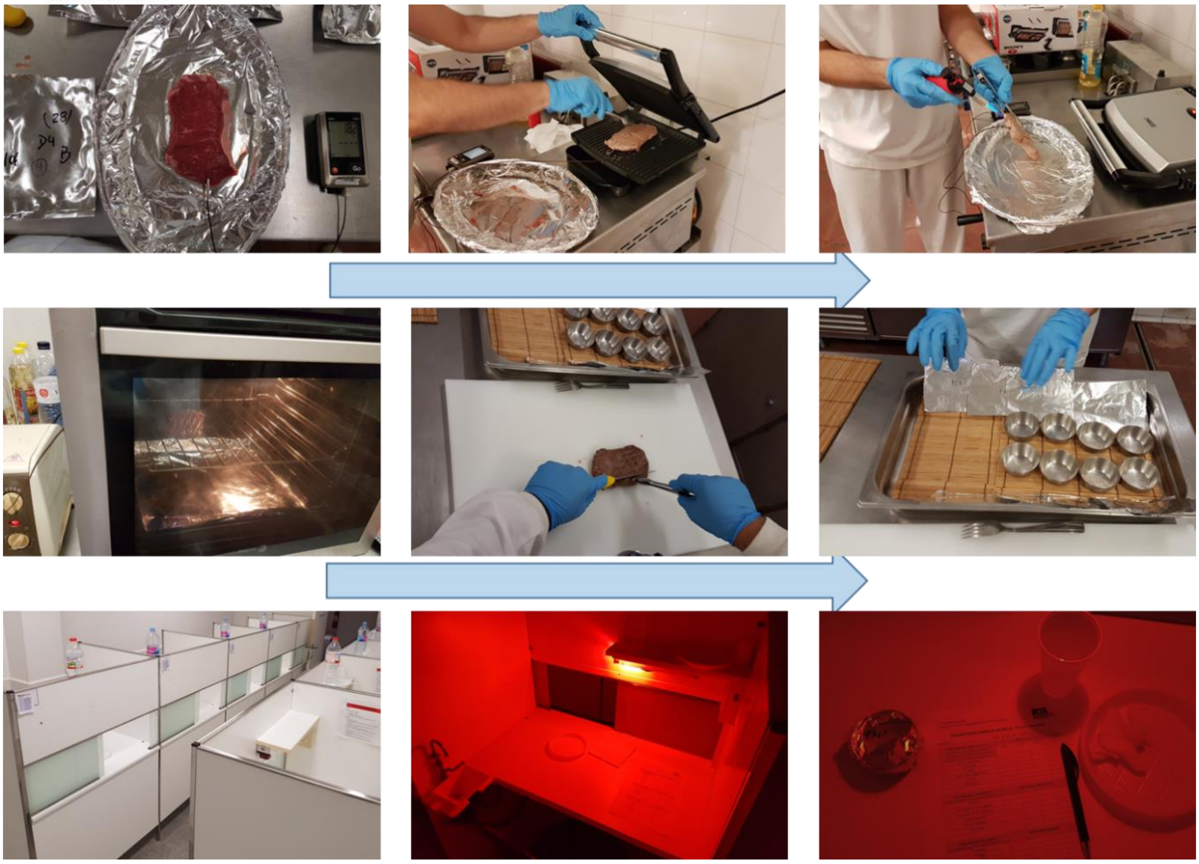


Figure 16. Cooking and servicing of aged samples flow chart

The panellists (n=8) were part of the trained panellists of the Quality Product Program from the IRTA. They participated in the training sessions with the objective of characterize specific attributes of the samples. After various training sessions, the attributes those were more detectable for all the panellist were chosen and punctuated at sensory sessions. These commons attributes are divided in three groups and are presented on Table 10.

Table 10. Aged meat attributes.

Odour traits	Texture traits	Flavour traits
Intensity	Tenderness	Beefy flavour
Beefy odour	Juiciness at 1^{st} bite	Liver
Liver	Juiciness at 5^{th} bite	Metallic
Lactic (butter like)	Chewiness	Acid
Sweet	Crumbliness	Lactic (butter like)
Aged	Fibrous felts	Aged

2.5 Statistical analyses

A descriptive analysis was performed using SAS ® software (SAS, 2001). Means, standard deviations, minimum and maximum values of every variable were calculated for each ageing method and ageing period. All data were analysed using analysis of variance with MIXED procedure. Ageing method (Dry or Tublin) and Ageing period (28 and 45 days) and their interaction were considered as fixed effect:

- Weight differences between ageing methods and ageing time were analysed with MIXED procedure, considering Ageing method as fixed effect.

- Colour values, colour preferences, purchase decision and shear force values were analysed with MIXED PROCEDURE considering Ageing method and Ageing time, as fixed effects, and its interaction was removed from the model when it was not significant. For these parameters, REPEATED statement (ageing time) was considered. In the particular case of Shear force analyses and cooking losses, the cooking day was included as blocking variable in the model.

Previously to the analysis of the sensory data, the scores obtained from all the panellists were standardized within animal and treatment. For this purpose, the STANDARD procedure of SAS was used with a mean of 0 and a standard deviation of 1. Furthermore, to facilitate the understanding of the measuring scales, the average for each animal and treatment was added to the standardized value.

The analysis of variance was performed with these transformed scores. The GLM procedure of SAS was used and the model included as fixed effect the type of ageing, the time of ageing and its interaction. The panellist within day was added to correct for differences between panellists due to the day of evaluation. The day of the sensory panel was added as blocking variable to correct for differences in the preparation of the samples between days.

Significance level was fixed at $P < 0.05$. Temperature and humidity profile analysis was performed with MATLAB software.

The model was:

$$\mathbf{Y_{ijklm} = \mu + A_i + T_j + (AxT)_{ij} + D_k + P_{kl} + e_{ijklm}}$$

Where,

Yijklm : observation ijklm

μ : mean of the model

Ai : Ageing Method (I = dry or highly water vapour permeable bag)

Tj : Time of ageing (j = 28, 45 days)

(AxT)ij : Interaction between ageing type and time of ageing

Dk : day of the panel evaluation (k = 1, 2)

Pkl : panellist within day (l = 1 to 8)

eijklm : error of the observation

3 RESULTS AND DISCUSSION

3.1 Temperature and Humidity assessment of the meat chamber

The assessment of MatureMeat ageing chamber allows to identify the variation in the parameters that were set to run the experiment. These variability in ageing conditions are seen as the variation in tone and level of intensity of the colours referred on the scales of the Figure 17. The difference between the set temperature and the temperature which the ageing samples received, is a useful information for the track of the ageing processes and this tool allows to identify corrective measures to be taken.

The figure also shows the percentage of the relative humidity (RH) which the samples are exposed to. The differences between set conditions reached levels around ± 5.0 ºC and the relative humidity is mostly around 50 – 60 %. This is largely different to the set conditions and certainly a factor of variability which affects samples to be aged. Having the knowledge of these parameters before starting ageing procedure allows to clarify about the number of samples, the position that may have used in the interior of the chamber and to ensure that the equipment is running correctly.

As shown by Juarez et al. (2010) higher ageing temperatures results in slight changes in quality traits of several muscles, as tenderness and may have negative impact on palatability attributes. Thus, endpoint conditions may have had double effect on ageing samples, regardless of the method and time of ageing.

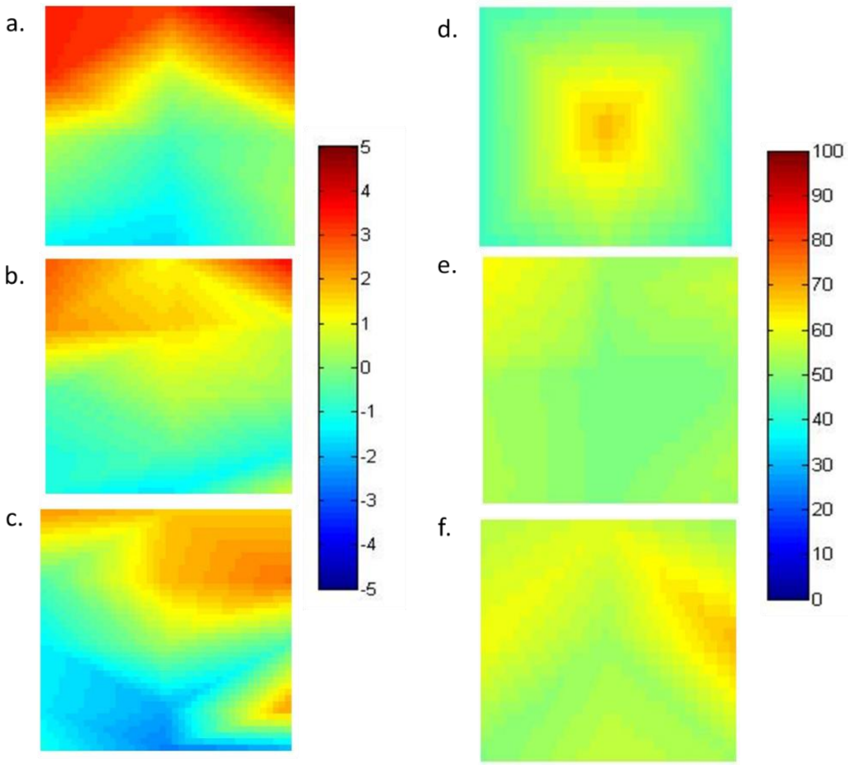


Figure 17. Temperature (ºC) and Relative Humidity (%) description of MatureMeat ageing chamber with actual running values. **a.** Temperature front level; **b.** Temperature middle level; **c.** Temperature back level; **d.** R.H. upper level; **e.** R.H. middle level; **f.** R.H. down level.

3.2 Weight losses

Table 11 shows the yield and weight losses from the two ageing methods in different stages of the experiment. Weight loss was higher in TB than in DA samples in the period 0 to 28 d, whereas there was no differences between methods in the period from 28 to 45 d. Berger et al., (2018) showed that samples aged in a high permeable bag had less weight loss, due to humidity and trimming losses, compared to dry ageing method. In the present study, trimming losses were significantly higher in TB samples than in DA samples (P = 0.0489) in the period 0-28 d. Accordingly to this, general yield and in addition, saleable yield, should be lesser in TB samples, although the difference disappeared from 0 to 45 d.

Table 11. Effect of ageing method dry (DA) and Tublin (TB) on weight losses, trimming losses, defrost losses and cooking losses.

	Method				
	DA		TB		
	LSM[a]	SE[b]	LSM	SE	P-value
Weight measurements (%)					
Weight loss 0-28d	11.56	0.53	21.99	0.53	<0.0001
Weight loss 28-45d	8.10	0.87	7.33	0.79	0.5327
Weight loss 0-45d[c]	19.38	0.77	27.60	0.71	<0.0001
Trimming losses (%)					
0-28d	54.00	1.94	60.16	1.94	0.0489
0-45d	62.95	1.39	62.74	1.39	0.9162
Defrost losses (%)					
t 0d	25.68	1.22	25.68	1.22	1.0000
t 28d	4.81	0.45	1.27	0.45	0.0002
t 45d	1.12	0.20	0.67	0.18	0.1282
Cooking losses (%)					
t 0d	25.37	1.19	25.37	1.19	1.0000
t 28d	32.33	1.62	18.26	1.62	0.0001
t 45d	1.73	1.71	1.28	1.56	0.0782

a LSM Least square means
b SE: standard error of the mean
c Estimated weight loss: average between weight loss 0-28d and weight loss 28-45d

Particularly, the exposed surface of TB samples was greater because the samples were deboned and the exposed lean tissue to the inner surface of highly permeable bag, this could be noted as an important factor of variability in time to conclude that these samples were more susceptible to humidity losses, and thus, remarkable weight loss. In contrast, fat tissue, bones and connective tissue of DA samples decreased the susceptibility to weight loss due to the protection that they offered, as seen by Smith et al. (2014).

Ahnström et al. (2006), Campbell et al. (2001) and Kim et al. (2017) have noted that weight loss values were lower in samples aged in high permeable bags compared to traditional ageing (DA) due to the reduction of interexchange of humidity. Even a great source of variability among these studies is the lack of standardized ageing conditions as temperature, relative humidity and air flow, the election of samples and its factors as breed, type of animal, conformation, fat score, e.g.; certainly, are critical points when the main is taking advantages of these novel bags, in contrast to the current study where factors as temperature, relative humidity, breed and conformation were similar.

Table 12 presents that there was an interaction between ageing method and time periods for cooking loss values. However, concerning about defrost losses, there was not significant difference (P> 0.05) neither between methods, nor in the interaction between method and time of ageing.

Table 82. Significance of the arrangement for defrost and cooking losses analysis.

	P-value		
	Method	Time	Method x Time
Defrost loss (%)	0.042	<0.0001	0.051
Cooking loss (%)	<0.0001	<0.0001	<0.0001

Defrost losses were not different when samples were frozen just before ageing method, as expected. Dry aged samples had higher defrost losses (4.81 %) compared to TB samples (1.27%) after 28 d of ageing, but after 45 d of ageing, no differences were observed between ageing methods. Opposite to results from Kim, et al., 2017 whom found that there were not effect of stepwise ageing (traditional ageing by 10 days and 7 days of ageing in a high permeable bag) and even found similar thawing loss (2.92 and 2.78 % respectively).

Cooking loss values reached 32.33% (DA) and 18.62% (TB) at 28 d, corresponding to the lack of available humidity due to the high percentage of drying from 0 d to 28 d period that lead to have a higher loss after being cooked. Also, these numbers have relation with some sensory factors that have been affected by the methods or time of ageing, that will be described in further sections.

In summary, for the weight measurements in the three described periods, the highest value of losses due to both, trimming and evaporation, were seen in TB packaged samples. Defrost and cooking losses were higher (P< 0.05) at 28 days of ageing in DA samples than in TB samples.

3.3 Instrumental meat quality

Table 13 shows that there was no interaction between method and period of ageing in all the instrumental measurements of colour. Lightness was affected by time of ageing but method of ageing did not cause a significant effect. Šulcerová,et al., (2017) advised that lightness of samples, measured by L* value, was affected in a significant way by ageing

time, resulting in a variability determined by the increasing and posterior decreasing of this value; this, is seen because of the changing pigment concentration (Aroeira et al., 2017).

Values of a* (redness) and b* (yellowness) were affected by method and time of ageing (Table 13). These values were lower at 28 and 45 d in TB than in DA samples. This result was unexpected because Dikeman et al. (2013) and Kim et al. (2016) stated that dry-aged beef steaks were slighter darker and had lower redness values compared with novel dry-ageing counterpart samples. The darker colour in the dry-aged beef should be mostly due to moisture loss and surface drying during ageing (Kim et al., 2017).

The interaction between method and time of ageing was significant for colour preference ($P = 0.039$) or tended to be significant for purchase decision ($P = 0.09$). The percentage of both measurements decreases in DA samples from 28 to 45 d, whereas it increases in TB samples.

Texture properties of DA and TB aged samples by the two time periods are shown in Table 13. The razor blade shear force did not show an interaction between ageing periods and methods. Method tended to affect ageing process ($P = 0.065$) but it was not possible to detect any effect from time of ageing, as expected. However, total work measured as area (g.mm), and calculated in the Warner Bratzler test, showed significantly differences among sampling periods. This result showed a decreasing on the value which positively indicates the degradation of muscle tissue and structural integrity by biochemical processes (Kim et al., 2017).

Also, the yield value represented by the values of the geometrical slope on the WB Shear Force test, that it measures the velocity of the cut, showed significant differences from the beginning to the other periods ($P< 0.0001$). Both, area and yield, seem to be affected by the effect of the day of sampling and testing. These results could explain because the total shear force value did not show significant difference between methods and ageing time.

Nonetheless, in the case of studies conducted by Ahnström et al. (2006), Berger et al. (2018), and Li, et al. (2013), the shear force values were no affected by ageing methods (traditional dry ageing and ageing in a high permeable bag). Smith et al. (2014) recorded values of 31 N as considerable tender range, which also are similar to the results obtained in the present study after 28 d of ageing (3.39 – 2.14 kg), but this similitude is only numerically appreciable.

Table 93. Least square means (LSM), standard error (SE) and P-value of defrost and cooking losses, colour parameters (CIE L*, a*, b*), colour preference, purchase decision and instrumental texture of traditionally dry aged (DA) and aged in a highly permeable bag samples (TB) after 28 and 45 days.

	Treatments														
	DA						TB								
Item	0 d		28 d		45 d		0 d		28 d		45 d		P-value		
	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	LSM	SE	M[1]	T[2]	MxT
Colour (CIE-Lab)															
L*	35.22	0.74	36.16	0.74	33.51	0.73	35.22	0.74	36.37	0.74	32.05	0.74	0.493	0.0001	0.479
a*	14.50	0.53	14.62	0.53	13.69	0.52	14.50	0.53	13.06	0.53	12.39	0.53	0.035	0.033	0.299
b*	14.25	0.41	14.9	0.41	14.05	0.4	14.25	0.41	13.81	0.41	12.09	0.40	0.007	0.009	0.071
a*/b*	1.02	0.02	0.98	0.02	0.97	0.02	1.02	0.02	0.94	0.02	1.03	0.02	0.796	0.097	0.190
Colour preference (%)	-	-	40.83	0.25	36.67	0.24	-	-	30.83	0.25	37.50	0.25	0.077	0.617	0.039
Purchase decision (%)	-	-	41.67	0.23	37.92	0.23	-	-	30.42	0.23	35.00	0.23	0.007	0.860	0.090
Instrumental texture															
Shear force (kg)	6.01	0.45	2.14	0.49	2.22	0.45	6.01	0.45	3.39	0.45	3.15	0.45	0.065	1	0.372
Area (g.mm)	61572	5458	24447	5996	25683	5458	61572	5458	36671	5458	33557	5458	0.155	<0.0001	0.545
Yield (g/mm)	818.7	52.44	275.92	57.61	291.88	52.44	818.7	52.44	451.92	52.44	379.26	52.44	0.063	<0.0001	0.278

[1] M = method of ageing

[2] T = time of ageing

3.4 Sensory quality

The effects of different factors studied on sensory attributes, divided in three groups (odour; texture; taste attributes) and its characteristic components of aged samples are shown in Table 14. Only few attributes were significantly affected by the interaction of ageing methods and time periods ($P < 0.05$): odour intensity and lactic odour, as well as juiciness at first and fifth bite. Nevertheless, traits as beef, lactic, sweet and aged odour, as well as crumbliness and fibrousnesses, were influenced ($P < 0.05$) by the effect of the cooking day and panel composition, which can explain a major percentage of variability in the results. Traits as toughness were influenced by the type of ageing method.

As Kim et al. (2017) showed, there were not significant interaction between stepwise method (dry ageing and ageing in highly permeable bag) and consumer liking and attribute intensities, resulting in no effect of ageing method and condition affecting sensory traits. On the other hand, current work showed the interaction of methods and time in few parameters that could be considered to be critic because of the importance they have between levels (odour, flavour, texture). Thus, this relation could show that ageing in a highly permeable bag can closely reproduce effects of traditional ageing, regarding to eating quality attributes and pleasantness.

However, in this study, a trained taste panel did not find the difference in flavour between treatments. In contrast, Campbell, et al., (2001) reported that wet-aged beef loins had significantly higher eating quality attributes, such as overall tenderness, juiciness, flavour, and overall palatability, compared to the loins from conventionally dry-aged beef carcasses.

In addition to flavour, a significant ($P < 0.05$) improvement in juiciness at 1st and 5th is reported in this work, matching results reported by Campbell et al. (2001), Ahnström et al. (2006), and Kim et al. (2017).

No differences were found in flavour traits between DA and TB samples ($P > 0.05$). These results were in agreement to those found by Berger et al. (2018) that also stated that overall liking scores were no affected by ageing treatments. On the other hand Šulcerová et al. (2017) stated that the time of ageing had significant effect for each monitored descriptors, except for the texture in bite, these results disagree the ones from the present experiment. This shows the great variability in ageing processes, even though when it seems to be development of novel procedures and developments.

Warren & Kastner (1992) found intensified flavour characteristics such as beefier and roasted flavour by dry-aged beef samples compared to wet aged or untagged beef. Particularly, steaks samples from traditional ageing and aged in a highly permeable bag had mostly equivalent sensory characteristics, but had remarkable higher scores compared to wet aged samples. This phenomena, may be attributed to the combined impact of the improvements of juiciness which subsequently results in a higher perception of the perceived tenderness (Berger et al., 2018).

Table 14. Least square means (LSM), standard error (SE) and significance of sensory properties of traditionally dry aged (DA) and aged in a highly permeable bag samples (TB) after 28 and 45 days.

	DA				TB				P-value			
	28		45		28		45					
	LSM	SE	LSM	SE	LSM	SE	LSM	SE	Method	Time	Method x Time	Cooking Day
Odour attributes												
Intensity	4.45	0.08	4.76	0.10	4.84	0.10	4.75	0.09	0.042	0.228	0.027	0.220
Beef	3.35	0.09	3.40	0.11	3.37	0.11	3.43	0.10	0.815	0.596	0.956	<0.0001
Liver	2.26	0.09	2.21	0.11	2.13	0.11	2.36	0.10	0.915	0.377	0.151	0.533
Lactic	2.64	0.10	2.95	0.12	2.71	0.12	2.54	0.10	0.117	0.533	0.027	0.018
Sweet	2.76	0.08	3.02	0.10	2.99	0.10	3.12	0.09	0.074	0.036	0.520	0.028
Aged	2.80	0.11	3.12	0.13	3.10	0.13	3.09	0.12	0.274	0.210	0.195	0.005
Texture attributes												
Toughness	3.58	0.11	3.26	0.13	3.66	0.13	3.82	0.12	0.011	0.511	0.062	0.077
Juiciness at 1st bite	3.73	0.09	3.96	0.11	3.17	0.11	2.55	0.10	<0.0001	0.072	<0.0001	0.246
Juiciness at 5th bite	4.72	0.12	5.05	0.14	4.30	0.14	3.26	0.13	<0.0001	0.010	<0.0001	0.073
Chewiness	4.40	0.12	4.37	0.14	4.60	0.14	4.55	0.12	0.140	0.732	0.931	0.266
Crumbliness	3.76	0.09	4.05	0.10	3.63	0.10	4.18	0.09	0.979	<0.0001	0.183	0.0001
Fibrousness	3.14	0.12	2.89	0.14	2.80	0.14	2.76	0.13	0.077	0.262	0.443	0.044
Taste attributes												
Beef	3.66	0.13	3.57	0.15	3.30	0.15	3.53	0.14	0.157	0.608	0.253	0.935
Liver	2.88	0.10	2.79	0.11	2.99	0.11	2.84	0.10	0.426	0.270	0.774	0.001
Metallic	3.03	0.11	3.04	0.13	2.88	0.13	3.06	0.12	0.602	0.466	0.487	0.840
Acid	2.92	0.12	2.79	0.15	2.80	0.15	2.91	0.13	0.977	0.953	0.384	0.712
Butter	2.71	0.09	2.95	0.11	2.63	0.11	2.62	0.10	0.053	0.260	0.253	0.704
Aged	2.78	0.12	3.11	0.15	3.08	0.15	3.34	0.13	0.056	0.035	0.785	0.008

CONCLUSIONS

1. The assessment of the ageing chamber leads us to clarify the actual conditions when meat is exposed to ageing process. The knowledge of these parameters allows us to determine corrective measurements.

2. Method of ageing had a significant effect on weight measurements, influencing the saleable yield and productivity.

3. Method of ageing affected redness and yellowness of meat samples. Colour characteristics were affected by ageing time with the possible impact on consumer perception about shelf life of product.

4. Method of ageing did not affect texture parameters but ageing time influenced some of them which are related to general pleasantness of consumers: total work on bite and velocity of cutting aged beef as well as perceived crumbliness.

5. Texture in mouth traits are affected by the interaction of ageing method and time, resulting in a significant variation of some sensory parameters perceived by a trained panel.

6. Under the parameters of the actual study, novel ageing in a high permeable bag had no advantages compared to traditional ageing.

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